general, mechanisms involved in particle release from platelets and erythrocytes appeared relevant to lymphocytes.

418-Pos Board B204
Effects of Cations on Phase Properties of Dipalmitoylphosphatidylcholine Assessed by Laurdan Fluorescence
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Two mechanisms have been proposed to account for the reduction in membrane fluidity observed in the presence of high concentrations of certain salts: direct binding to phospholipid molecules and dehydration of the membrane. To address these proposals experimentally, we prepared dipalmitoylphosphatidylcholine liposomes with laurdan, which is sensitive to the presence and mobility of water molecules in the bilayer. Laurdan emission spectra and steady-state anisotropy were then acquired simultaneously at multiple temperatures below and above the main phase transition. Calcium ions (1 M) raised the transition temperature by 8.5 K with minimal effects on the apparent transition cooperativity and ΔH. Moreover, laurdan spectra (quantified by generalized polarization, GP) were blue-shifted at all temperatures (by 0.05-0.1 GP units) confirming modest dehydration of the membrane. Anisotropy reflected only the effects on the phase transition with no alterations to values at the temperature endpoints suggesting that the mobility of laurdan was unaltered by calcium. In contrast, sodium ions produced little change to the transition temperature, but reduced both the apparent cooperativity and ΔH (by ~25%). The laurdan GP was identical to control samples in the lipid gel phase, but elevated by more than 0.1 GP units at temperatures above the phase transition, suggesting that dehydration by the salt only occurred in the fluid phase. Surprisingly, anisotropy was lowered at all temperatures by sodium, reflecting greater mobility of laurdan notwithstanding the negative impact of the ion on overall membrane fluidity. Effects of potassium on the phase transition were similar to those of sodium, but no change in either GP or anisotropy were observed at the endpoint temperatures. These results argue that the mechanism of salt effects on membrane properties is more complex and ion-specific than previously hypothesized.

419-Pos Board B205
2H Solid-State NMR Studies of the Antimicrobial Peptide MSI-78 Interacting with the Membranes of Whole Escherichia Coli
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Antimicrobial peptides (AMPs) are ubiquitous molecules that can display antimicrobial activity against bacteria, viruses, protozoa and various other pathogens. A key aspect of AMP activity is their interactions with biological membranes. Solid-state NMR spectroscopy has thus been an important tool for their study. Previous experiments, on model membrane systems, have elucidated important aspects of AMP mechanism. However, the extent to which actual in vivo AMP activity can be understood from model studies is necessarily limited. Peptide-membrane interactions under physiological conditions are presumably influenced by additional factors such as: interactions with lipopolysaccharides, the presence of membrane proteins, membrane compositional heterogeneity, lipid domains, etc. In order to bridge the gap between the NMR studies of AMPs using model membranes and the AMP-membrane interactions occurring in intact cells, we have designed a procedure to incorporate high levels of 2H-NMR labels, specifically into the cell membrane, by creating a novel strain of E. coli: LA8. Using this strain we are able to reproducibly quantify the effects of the AMP MSI-78 on lipid chain order in bacterial membranes. Treatment with MSI-78 led to an increase in the disorder of the bacterial membrane. This was observed by the decrease in the average order parameter and by the increase in intensity at the lower frequencies. The peptide:lipid ratios needed to observe MSI-78's effects on acyl chain order in the intact cells falls between the ratios required to observe effects in NMR studies of model lipid systems and the ratios required to observe inhibition of cell growth in biological assays.

420-Pos Board B206
Investigation of the Role of Cholesterol Superlattice in Release Kinetics of Drugs from Stealth Liposomes
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The development of liposomal drug formulations to combat diseases such as cancer is a growing field of study, with over twenty liposomal chemotherapy drugs currently on the market or undergoing clinical trials. We have previously demonstrated that cholesterol, often employed as a membrane stabilizing agent in liposomal drugs, plays a delicate and critical role in the release kinetics of the chemotherapy drug combretastatin A4 disodium phosphate (CA4P) in accordance with the principles of sterol superlattice. Here, we investigate the impact of membrane cholesterol content on the release of the same drug from "stealth" liposomes, which possess a polyethylene glycol (PEG) coating that renders them nearly invisible to the immune system and allows more efficient targeted drug delivery. Samples of stealth liposome-encapsulated CA4P were prepared in which the cholesterol content differed little (0.4 mole%) between tubes in order to assess the effect of cholesterol content on a fine scale. First, we used a fluorescence assay which exploits the intrinsic fluorescence of CA4P to determine its release kinetics from liposomes in aqueous solution. Next, a cytotoxicity assay was employed to determine the effect of the various liposomal drug formulations on cancer cells in vitro. Finally, the data were correlated with the predictions of the theory of sterol superlattice and compared to previous experimental results.

421-Pos Board B207
Study of the Interactions Between Model Membranes and a Truncated Hemoglobin (trHbN) by NMR and Infrared Spectroscopy
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Approximately one third of the world population is infected by the pathogenic bacterium Mycobacterium tuberculosis. A key to the resilience of M. tuberculosis resides in part in its capacity to enter a latent state where it can resist different oxygen and nitrogen oxidative species such as hydrogen peroxide, nitric oxide and peroxynitrite produced by the infected macrophages. One protein responsible for that is trHbN, a truncated hemoglobin, that detoxifies nitric oxide from the cellular environment. It is thought that the protein heme achieves that through this mechanism (Mishra et al, J. Am Chem. Soc., 132, 2968-2982):
Fe(II)-O2 + NO* –> Fe(III) + NO3-
The importance of studying this protein lies in the fact that we now have to deal with new antibiotic-resistant strains. Therefore, we have investigated the trHbN orientation and conformation in different lipid model membranes. We also have studied the effect of the protein on these membranes. FTIR was used to observe changes in the conformational order of the lipids in the presence of the protein and the protein secondary structure. Furthermore, solid-state NMR provided information on the membrane conformation and on the protein orientation. These studies were performed in pure lipids, and also in a mixture of two different lipids (TOCL and DOPE) which was optimized to achieve a composition similar to that of the bacterial membrane.

422-Pos Board B208
Using Super-Resolution Fluorescence Localization Imaging to Probe Raft Hetergeneity in Fixed and Live Cells
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We are using super-resolution fluorescence localization imaging to probe the organization and mobility of 'raft' and 'non-raft' markers in the plasma membranes of mammalian cells. Our goal is to experimentally test our recent predictions regarding protein organization and mobility in membranes containing critical fluctuations that are coupled to cortical cytoskeleton (1). Our raft constructs include the transmembrane domain of linker of activated T cells (LAT-TM) the transmembrane domain of influenza hemaglutinin protein (HA-TM), GPI-linked proteins, and cholera toxin B subunit bound to the ganglioside GM1. Our non-raft markers include palmitoyl-null mutants of LAT-TM and HA-TM constructs, and the lipid probe $DiIC_{12}$. We are probing the organization and mobility of the above markers either through conjugated photoactivatable (PA) fluorescent proteins (PALM), or through the reversible blinking of organic fluorophores (STORM). By measuring autocorrelations and cross-correlations from one and two color fixed cell images, we quantitatively probe the nano-scale organization of components and can compare our findings to our recent predictions. We quantitatively probe the diffusion and confinement of membrane components by measuring the mean

squared displacement (MSD) of single molecule tracks obtained from live cell measurements. Exploiting the photo-switching of PA fluorescent proteins and blinking organic fluorophores, we detect an ensemble of single molecules in each cell investigated, and can analyze populations of diffusers with incredible statistics. By comparing theoretical predictions with quantitative experimental observations, we aim to test our working hypothesis that critical composition fluctuations provide the physical basis of raft heterogeneity.

1. Machta, B.B., S. Papanikolaou, J.P. Sethna, and S.L. Veatch, 2011. *Minimal model of plasma membrane heterogeneity requires coupling cortical actin to criticality.* Biophys J. 100: 1668-77.

423-Pos Board B209

Phosphoinositides Alter Lipid Bilayer Properties

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Phosphoinositides are involved in cell-signaling pathways that regulate vital cell functions such as membrane excitability and trafficking, and cell metabolism, motility and proliferation. At the plasma membrane, phosphatidylinositol-4,5-bisphosphate (PIP_2), which constitutes approximately 0.25% of cell phospholipid, is a key messenger in membrane-delimited signaling. PIP_2 regulates structurally and functionally diverse membrane proteins including voltage- and ligand-gated ion channels, inwardly rectifying ion channels, transporters and receptors. The mechanism(s) by which PIP_2 regulates many of its various ''receptors'' remain to be elucidated. Here we explore the notion that the amphiphilic phosphoinositides, by adsorbing to the bilayer/solution interface, alter bilayer properties such as curvature and elasticity. Such changes in bilayer properties can alter the equilibrium between membrane protein conformational states and thereby alter function. Taking advantage of the gramicidin channels' sensitivity to changes in the lipid bilayer properties, we used fluorescence-based and single-channel gA assays to examine the effects of (diC8) phosphoinositides -PI, $PI(4,5)P_2$, $PI(3,5)P_2$, $PI(3,4)P_2$ $PI(3,4,5)P_3$ as well as long-chain $PI(4,5)P_2$ on the lipid bilayer. The diC8 phosphoinositides, except for $PI(3,5)P_2$, alter lipid bilayer properties with potency that decreases with increasing charge. Among the long-chain $PI(4,5)P_2$s, the naturally occurring 1-stearyl-2-arachidonyl-$PI(4,5)P_2$ is a more potent bilayer modifier than di-oleoyl-$PI(4,5)P_2$. The diC8 and the naturally occurring $PI(4,5)P_2$ have similar effects on short and long gA channels, indicating that changes in bilayer curvature dominate over those on bilayer elasticity. In contrast, diC8PI, which was more bilayer-active than diC8PIP_2 altered bilayer elasticity. Our results show that application of exogenous PIP_2 and its structural analogues (with changes in acyl chain length or phosphorylation state) alters lipid bilayer properties. These PIP_2 lipid bilayer effects may be important for some of the many different effects on membrane protein function.

424-Pos Board B210

Direct Observation of Plasma Membrane Domains using Super Resolution Microscopy

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The composition of the plasma membrane has long been modeled as a mosaic fluid. However, in the last few years there has been evidence that suggests the plasma membrane to be a dynamic and highly compartmentalized structure. This organization in domains results in a differential spatial distribution of signaling proteins on both leaflets of the plasma membrane. It is still debated whether inner and outer leaflet domains are linked. The lateral segregation of membrane proteins plays a role in cell signaling and protein-protein interaction. Thus, it is of high scientific interest to further investigate these domains.

The Ras protein resides on the inner leaflet of the plasma membrane. Here we used super-resolution microscopy to study the compartmentalization of H-Ras and its membrane anchor CAAX, fused to the photo convertible dye Dendra2. The signal of single Dendra2 molecules is recorded and statistical analysis is applied to localize these molecules. On the apical membrane of 3T3 fibroblast, domains of 150nm were detected for both the full protein and its membrane anchor. To investigate a possible link between inner and outer leaflet domains, cells where treated with Choleratoxin B (CtxB). This leads to clustering of the outer-leaflet ganglioside GM1. Neither size nor the amount of domains were dependent on incubation with CtxB. However, incubation with CtxB did lead to an increase in H-Ras density inside the domains, indicating a connection between lipid organization on the outside and protein distribution on the inside of the plasmamembrane.

425-Pos Board B211

Statins Modify Lipid Bilayer Properties

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Statins are drugs that are widely prescribed to manage hypercholesterolemia. Statins exert their primary mechanism of action by inhibiting the HMG-CoA reductase, thus preventing cholesterol synthesis. In addition to this canonical action they also alter the function of diverse membrane proteins. Because statins are amphiphiles that modulate the function of different, structurally unrelated membrane proteins, we investigated whether statins could alter lipid bilayer properties at concentrations where they alter membrane protein function. To this end, we used the gramicidin-based fluorescence assay (GBFA) as well as single-channel electrophysiology. We found that atorvastatin, fluvastatin, lovastatin, mevastatin, pravastatin, and simvastatin all increased the rate of fluorescence quenching, meaning that they shifted the gramicidin (gA) monomer dimer equilibrium toward the formation of conducting dimers. Statins thus alter lipid bilayer properties, with fluvastatin being the most active and rosuvastatin the least active. When examined using single-channel electrophysiology, simvastatin, pravastatin, and fluvastatin increased the lifetime and appearance rate of gA channels with fluvastatin being the most active and pravastain being the least active. We observe larger effects on the shorter channels; the hydrophobic mismatch dependant effects indicate a change in bilayer elasticity. We conclude that statins alter lipid bilayer properties by a common mechanism, through an increase in bilayer elasticity, and that specific channel-statin interactions are not the sole mechanism of action for statins.

426-Pos Board B212

Heterogeneity of Water Dynamics of Hydrated Lipid Bilayers in Atomistic MD Simulations

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Probing dynamics of water molecules interacting with polar headgroups of hydrated lipid membranes is vital in understanding general properties of membrane systems. Recent terahertz spectroscopy experiments provided new insights into dipolar relaxation and dynamics of water molecule reorientation in lipid bilayers with decreasing hydration level[1]. We perform molecular dynamics simulations of DOPC with varied levels of hydration. Our simulation models reproduce the experimental therahertz spectroscopy results with reasonable accuracy. Previously, three different types of water molecules were proposed that were described as irrotational water, bulk water, and fast water with distinct relaxation dynamics. We analyze single molecule dipole correlations in detail to study reorientational dynamics of water molecules in our simulated systems. Our results provide us with distributions of relaxation properties as a function of hydration level. We identify a population of water molecules which are tightly bound to lipid headgroups and exhibit relatively very slow relaxation dynamics. The remaining water molecules in the simulated systems, whose reorientational dynamics can be probed on the timescale of our simulations exhibit a broad heterogenous distribution of dynamical properties. This result suggests that models used to interpret experiments probing the reorientational dynamics of water molecules in a hydrated lipid bilayer should be based on a proper description of this distribution instead of isolated populations of water molecules with distinct properties.

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427-Pos Board B213

Understanding Plasma Memebrane Organization and Cellular Homeostasis Relationship

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